## Remarks

Claims 1-19 were present in the application as filed. By preliminary amendment filed with the initial application papers, claim 6 was canceled and claim 20 was added, thereby resulting in pending claims 1-5 and 7-20. Claims 1-5 and 7-20 remain pending in the application. Claims 1-5 were amended in a paper that was filed contemporaneously with the filing of a Request for Continued Examination under 37 C.F.R. §1.114 to remove the finality of a final Office Action mailed July 7, 2003. Claim 20 is canceled above. Claims 1-5 and 7-19 remain pending in the application.

Claim 1 is amended herein to clarify the nature of the ionic surfactant used in the claimed method. Accordingly, claim 1 recites a method comprising treating a pharmaceutical drug or vaccine with a concentration of an ionic surfactant *that is effective to dissociate the endotoxin from the amphiphilic pharmaceutical drug or vaccine without adversely affecting the properties of the drug or vaccine including its ability to be retained by a filter.* Directly thereafter, the solution is filtered through a molecular weight cut-off filter having a pore size effective to retain the amphilphilic pharmaceutical drug or vaccine but allow the dissociated bacterial endotoxin to pass therethrough. Additionally, claim 1 is amended to indicate that following filtration, *the amount of ionic surfactant remaining in said solution is less than 0.01%.*

Support for the amendment is found in the specification on page 6, second full paragraph and page 17, first full paragraph.

### Claim Rejection Under 35 USC §103(a)

Claims 1-5 and 7-20 remain rejected as being unpatentable over Shanbrom (EP 0 083 999) in view of Shanbrom (U.S. Patent 4,315,919).

In view of the above amendments, Applicants' believe that the combination of Shanbrom (EP 0 083 999) and Shanbrom (U.S. Patent 4,315,919) does not teach the claimed invention.

The depyrogenation method taught by Shanbrom ('919) uses a non-denaturing amphiphile, including ionic and non-ionic surfactants, in conjunction with protein precipitation methods to remove endotoxin from biological and pharmaceutical products. The reference, however, only provides evidence with respect to the **non-ionic** surfactant, Triton-X 100 (See Examples 1-5). Furthermore, Shanbrom ('919) additionally contains a disclaimer with respect to the use of some ionic surfactants, such as sodium deoxycholate. Shanbrom further discloses that amphiphiles are effective in removing endotoxin when used to destroy the endotoxins prior to their separation and removal along with the amphiphile in the supernatant (col. 4, lines 20-29).

Shanbrom (EP) teaches depyrogenation of an aqueous albumin solution using a **non-ionic** surfactant followed by filtration with a 10kd MWCO filter. Interestingly, endotoxin is not detectable in either the retentate or the permeate.

Neither Shanbrom reference discusses the level of residual surfactant remaining in the final product.

Applicants' invention provides a method of removing bacterial endotoxin from an amphiphilic pharmaceutical drug or vaccine. In one embodiment of the present invention, the the vaccine is a viral protein antigen. Protein antigens present special challenges with respect to endotoxin removal since 1) due to both having amphiphilic structures, the antigens and endotoxin become strongly associated under aqueous conditions and 2) the native conformation of the antigen must be preserved.

For viral antigens like influenza virus hemagglutinin (HA), which occur in complexes called "rosettes", maintenance of the quarternary structure of the antigen is important and the challenge is to dissociate the endotoxin from the drug or vaccine without adversely affecting the properties of the drug or vaccine, including its ability to be retained by a filter with a MWCO cut-off based on the original molecular weight of the vaccine to be filtered.

Applicants' solution to this problem is to treat the process solution with an ionic, preferably anionic surfactant, which is able to dissociate the endotoxin from the amphiphilic drug or vaccine substance without altering the structure of the drug or vaccine itself. Inherent in the trimeric structure of influenza HA antigen rosettes, for example, is its molecular weight and concomitantly, its ability to be retained by the filter. The resulting solution is then subjected to ultrafiltration such that the larger amphiphilic drug or vaccine complex is retained by the filter, while the smaller, dissociated endotoxin fragments and surfactant pass through the filter.

As previously discussed, therefore, the deficiencies of Shanbrom (EP) are not cured by the addition of the teachings of Shanbrom ('919), since neither reference teaches that the properties of the protein treated are not adversely affected by the treatment. Without such teaching, the claims as amended herein, cannot be obvious.

For all the foregoing reasons, Applicants submit that the claimed invention is clearly distinguished from the prior art and respectfully request that the rejection under §103 be withdrawn.

It is respectfully submitted that the above-identified application is now in condition for allowance and favorable reconsideration and prompt allowance of these claims are respectfully requested. The dependent claims are believed allowable for the same reasons as the independent claims from which they ultimately depend, as well as for their additional limitations. Should the Examiner require clarification of any of the above, the Examiner is invited to contact Applicants' undersigned attorney at the telephone number listed below.

Respectfully submitted,

Date: May 18, 2004

Kathy Smith Dias
Attorney for Applicants
Registration Number 41,707
HESLIN ROTHENBERG FARLEY & MESITI P.C.
5 Columbia Circle
Albany, New York 12203
Telephone: (518) 452-5600
Facsimile: (518) 452-5579